

R E M A R K S

Claims 3, 24, 25, 29, 30 and 34 have been cancelled and in Claim 58 a typographical error has been corrected. The claims have further been amended to delete the possibility of R⁴, R⁵ or R⁶ being carbohydrate or X³ being S-alkyl .

Turning first to the rejection under 35 USC 112 and the application of the Wants factors:

(A) Breadth of the claims:

Claims 1-6, 8-15, 18, 24, 25, 29-40, 44-47 and 51-59: have been amended to delete claims on carbohydrates and X³ = S-alkyl. The examiner's comment on X² as S-alkyl was not understood since this does not seem to be encompassed by the claims. ,Deletion of coverage of carbohydrates has been made to expedite prosecution even though the applicants believe that the carbohydrates serve as a pro-drug, i.e. they degrade in the stomach to release the parent flavone compound. The method of treatment claims have also been amended to delete reference to treatment of septic shock and cancer

Cytokines are involved in a very important physiological role inside the human body. Any overproduction can lead to many undesirable complications. Therefore, inhibition of TNF- α overproduction can have many applications (see Attachment 1- an explanation of this issue prepared by one of the applicants, Dr Edwin S. C. Wu). Some of the applications are proven clinically and some remain to be explored. In drug discovery, our clinical prediction is often based on the sound rationale and then move the compound to clinical trials. That is how a drug company achieves the discovery of a new drug in a entirely different mechanism and different disease. A drug company can not afford to test clinically before it can get a patent for treating certain diseases.

(B) The level of predictability in the art. People who are skilled in the art would say that Lee's et (WO 01/30342) patent application covers six groups of *structurally unrelated compounds* (the common features between those eight compounds are an aromatic group and a sugar): i) flavone compounds (Fig. 1B, 19B) and a flavone glycoside (Fig. 19A) which is expected to serve as a prodrug in vivo; ii) Wogonon (Fig. 19C), a flavone compound, but it has substitutions at 5,7, and 8-positions while baicalein and oroxylin A have substitutions at 5,6, and 7-positions. People who are skilled in art of SAR (structure-activity relationship) can not predict if they will be the same because they are positional isomers unless a pharmacophore is identified; iii) flavonol compound, myricitrin (Fig. 1A) is a flavonol glycoside and should be considered a separate entity because of the presence of a 3-hydroxyl group even we assume that the glycoside is a prodrug of flavonol ; iv) a simple sugar ester (Fig. 1C); v) a complex aromatic esters containing two sugar unit (Fig. 1D to 1F); vi) anthraquinone derivatives (Fig. 1G and 1H) which is quite different from flavones structurally. It is so obviously that they do not have a common pharmacophore and thence they lack any predictability based on the principles of medicinal chemistry.

In present application case, based on the TNF- α results of 5,6,7-trialkoxy substituted flavones or their 4'-substituted analogs "submitted previously", one skilled in the art would reason that a pharmacophore exists: it requires the presence of 5,6,7-trisubstituted akyo group and a hydroxyl group or a hydrogen bonding group on either of the 5,6, or 7 position or 4'-substituted side chain will enhance the TNF- α inhibition. Our approach in structural variations or modifications based on 5,6,7-trihydroxy or trialkoxychromones is considered as a rationale one with a gradual change in structures based on the principle of SAR or medicinal chemistry. Phosphates or sulfates of phenols are known to act as prodrugs.

To further illustrate the point, Edwin Wu, one of the inventors in this application obtained four US patents on antihypertensive chromonoxypropanolamines, i.e. derivatives of chromones including flavones, isoflavones, 2,3-diphenylchromones and aurones (US 4,495,198 Antihypertensive chromonoxypropanolamines; US 4,668,804 Chromones; 4,668,805 Chromones; US 4,806,660: Aurone oxypropanolamines). All the compounds exhibited antihypertensive activity *in vivo*. Apparently, the pharmacophore involves in those four closed related series of compounds is the presence of an aromatic ring plus a oxypropanolamine.

(C) The existence of working examples. Similar to the sulfate of baicalein 11a, the testing results on baicalein indicated the inhibition of TNF- α (TNF-alpha) and superoxides as shown in the data set out in the figures accompanying the present application. In contrast to the Examiner's belief that there is no correlation between the levels of TNF- α , superoxide anion, plasma nitrate and iNOS in the treatments of any diseases, there are working examples as specified in the specification of this application that (p.4 line 16-30), TNF- α inhibitors such as Remicade (infliximab) and Etanercept (Enbrel) have been clinically used in the treatment of rheumatoid arthritis, Crohn's disease, etc because excessive production of TNF- α in those diseased patients. As also stated in our specification (line 25-30 of p. 4) that animal studies in association with studies conducted in humans indicate a potential role for TNF modulation in Crohn's disease...insulin resistance, ... multiple organ failure, pulmonary fibrosis, and atherosclerosis. Similarly, the specification also covers role of superoxide anion radical. Additional literature (attachment 1) is attached for your review. In drug exploratory discovery, we can only base on either *in vitro* or *in vivo* data to predict possible human efficacy, unless the correlation exists between *in vitro* or *in vivo* data and human efficacy. In later case, it is no longer an exploratory research because we are working on known mechanism related to human disease.

Elevation of liver enzymes such as GPT or GOT is an indication of liver damage and used clinically to diagnose the liver. A copy of Merck Manual is also attached in attachment 1 for your review.

(D) The quantity of experimentation. The animal studies are expensive and we have demonstrated similar activities between Compound 11a and baicalein. To reduce the animal studies, we started to use *in vitro* assay to estimate the efficacy of each compound. As shown in the response of September 2007, a SAR is shown in the Table of TNF- α which enables one skilled in the art to pin-point the crucial pharmacophore. This pharmacophore allows one skilled in the art to predict which substituents may affect the activity. Since the applicants do not alter the parent structure of 5,6,7-trihydroxy or alkoxychromone, all additional substituents will be expected to modify the activity to a certain extent, either enhance or diminish the parent compound's efficacy or activity.

It is therefore submitted that the claims as now presented comply with the enablement requirements of 35 USC 112 first paragraph.

Turing now to the written description requirement of the same paragraph of 35 USC 112, It is submitted that the definition of "wherein n is 0 or 3", is inherent in the original disclosure in that there are many compounds synthesized and two examples on p. 29 are given here:

compound 1d (4',5,6,7-tetrahydroxyflavone) satisfies n=0 Y =OR⁴ where R⁴ is H

compound 1c (4',5,6,7-tetramethoxyflavone) satisfies n=0 Y =OR⁴ where R⁴ is CH3.

Regarding to "substituted phenyl", there are many compounds have substituted phenyl, for examples, all compounds on p. are with 4'-substitution.

Regarding to “X3 is ... OR1”, it is exemplified in compounds 1c and 1d.

Regarding to “OR1 is O(CH₂)_nY, wherein n is 1 or 2, Y is OR4...” and “n=0-3” (claim 1), they are exemplified on in compounds on p. 34 and 35:

4’-(Carbethoxymethylamino)-5,6,7-trimethoxyflavone.

4’-[N-methyl-N-(3-methoxypropyl)amino]-5,6,7-trimethoxyflavone,

4’-[N,N-di(2-hydroxyethyl)-amino]-5,7-dihydroxy-6-methoxyflavone and 4’-(2-hydroxyethylamino)-5,7-dihydroxy-6-methoxyflavone,

4’-(2-methanesulfonatoethylamino)-5,7-dihydroxy-6-methoxyflavone,

4’-[2-(N,N-diethylamino)ethylamino]-5,7-dihydroxy-6-methoxyflavone

Regarding to “combination thereof” (Claim 18), we could not locate the phrase ““combination thereof” in Claim 18. It was in the original claims.

It is therefore submitted that the definitions now set out do not add new matter and are fully in conformity with the original written description, thereby complying with the requirements of 35 USC 112.

As noted above, the method of treatment claims have been amended to delete reference to septic shock and cancer. These deletions make clear the difference between the present invention and the disclosure of Lee, which the examiner suggests may indirectly refer to septic shock and Handler, whose brief reference to compounds being “antileukemic” might be interpreted as teaching treatment for cancer. Although the applicants do not necessarily agree with the Examiner’s position on these matters, the points of possible overlap have been deleted in order to expedite prosecution. It is therefore submitted that all of the method of treatment claims are now clearly allowable over the cited art.

Turning now to the rejection of compound Claims 1 – 6 under 35 USC 102(b), Claim 1 contains novel structures which are not exemplified by Cassels et al (US Patent No. 5,756,538). Cassels et al disclose the preparation of 2'-halogenated chrysin [(5,7-dihydroxy)-flavone]. This patent does not teach 5,6,7-tri-hydroxy-, trialkoxy-, or 4'-substituted-5,6,7-trihydroxy- or alkoxy-flavone, isoflavone, and diphenylchromone derivatives. Cassels et al do not prepare any unhalogenated compounds and nor do they show any paper examples of those compounds. Some combination of these substituents are chemically infeasible to be synthesized. For example, when all the substituents are NH₂ or NO₂. Those skilled in the art of SAR would not expect that unhalogenated compounds will have the same potency or efficacy as those of halogenated chrysin analogs., for example chloramphenicol, a chlorinated antibiotic, and DDT, a chlorinated pesticide.

Henders et al (US Patent No. 6,541,613) disclose a series of esters of 7-hydroxyisoflavones, 4',7-dihydroxyisoflavones, 6,7-dihydroxyisoflavones, and 4',5,7-trihydroxyisoflavones (column 3, line 1-15) The true meaning of the disclosure is, however, very unclear. Claim 1 of Handler requires that at least one if the groups corresponding with the OR¹, OR² or OR³ groups or when X² is ArX³T the X³T group is a ZOOO group or a ZPO₄ group, Z being defined as alkyl, alkenyl, alkynyl, alkoxyalkyl, alkylthioalkyl, amino alkyl cycloalkyl, aryl, aralkyl or alkylaryl. Z is not defined as a hydrogen. These are not possibilities covered by the applicant's claims. Handler's disclosure does refer to other compounds. Many of these have carbohydrate substituents that are now removed from the scope of the applicant's Claim 1. So far as other possible substituents of the phenyl group are concerned, it does not seem that any of these fall within the definition of ArX³T now set out as being a possibility for X² of the present claims. In any case, the compounds are referred to without any support and with unpredictability as to the properties of these compounds.

Furthermore, Handler does not exemplify the preparation of 5,6,7-trihydroxy-, trialkoxy-, tetrahydroxy-, or tetraalkoxy-isoflavones, although they claim so many unrelated compounds. They do not teach 4'-substituted isoflaones, 4'-substituted 5,6,7-trihydroxy-, and trialkoxy-flavones or diphenylchromones. Some of the claimed compounds are impossible to make, for examples when all substituents are ArCOO-, ArPO₄-, c-HexCOO- or c-HexPO₄- due to steric hindrance.

Lee et al., WO 01/30342) application does not provide any enabling disclosure of compounds according to the present claims. Lee et al., describe six distinctively different structural classes of compounds, as described above and does not teach any SAR because of diversity in structures, thus unpredictability in activity, as the Examiner stated in the Office Action. Lees's disclosure of compounds in Formula 1 is without support and beyond predictability and the principle of SAR. They do not exemplify any preparation of any claimed compounds (pages 15-16). Synthesis of these compounds are not obvious to people who are skilled in the art of organic synthesis and some compounds may not be possible to synthesized, for example, R3, R4, R5, and R6 = biaryl. Although they claim so many unrelated compounds, they do not teach 4'-substituted isoflaones and diphenylchromones. Formula 1 covers 8-substituted and 2'- or 3'- substituted flavones, while we do not. In the flavone series, they do not claim phosphate and sulfate esters. The following 4'-substituted flavones are not covered in Formula 1 of Lee et al's application and are new compounds:

4'-(methylsulfonamido)-5,6,7-trimethoxyflavone (10e)

4'-[N-methyl-N-(3-methoxypropyl)amino]-5,6,7-trimethoxyflavone,

4'-[N,N-di-(2-hydroxyethyl)-amino]-5,7-dihydroxy-6-methoxyflavone,

4'-(2-hydroxyethylamino)-5,7-dihydroxy-6-methoxyflavone,

4'-(2-methanesulfonatoethylamino)-5,7-dihydroxy-6-methoxyflavone,

4'-[2-(N,N-diethylamino)ethylamino]-5,7-dihydroxy-6-methoxyflavone,

4'-(methylsulfonamido)-5,6,7-trimethoxyflavone,

4'-[2-(N,N-diethylamino)ethoxy]-6,7-methylenedioxy-5-hydroxy-flavone,

4'-(2,3-dihydroxy-propyloxy)-5,6,7-trimethoxyflavone,

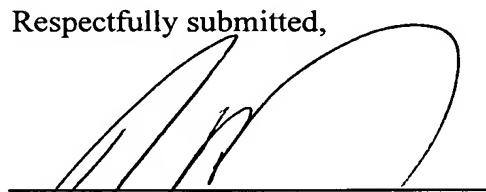
4'-(Carbmethoxymethoxy)-5,6,7-trimethoxyflavone.

Lee et al., state in the abstract that "The present invention is also directed to a method of activating K⁺ channels in mammals; as well as methods for treating septic shock, for inhibiting expression of angiotensin converting enzyme, for treating or preventing aneurysms and for reducing inflammation and related pathological changes using these compounds." We therefore deleted our claims on septic shock. Although TNF- α is implicated in the sepsis, this application does not teach the use of TNF- α inhibition in the treatment of organ damage and other diseases.

Regarding to the inventor Edwin Wu, he contributed to the idea of isoflavone and diphenylchromone analogs at the time of filing PCT application, Nov. 2003.

It is submitted that this application is now in order for allowance and an early action to this end is respectfully solicited.

Respectfully submitted,



JOHN RICHARDS
C/O LADAS & PARRY LLP
26 WEST 61ST STREET
NEW YORK, NEW YORK 10023
REG. NO. 31, 053 (212-708-1915)

ATTACHMENT

1) Diseases associated with TNF-a

There is evidence that specifically blocking TNF- α alleviates symptoms and sometimes halts pathology associated with inflammatory diseases. Therapeutics such as etanercept and infliximab that specifically inhibit TNF- α are used clinically to treat rheumatoid arthritis (RA), Crohn's disease, ankylosing spondylitis, psoriatic arthritis (1-6). Treatment of RA with TNF- α -specific inhibitors has been demonstrated to ameliorate joint damage and sometimes promote joint healing in (7-8).

Pro-inflammatory cytokines, for example tumor necrosis factor (TNF)- α , can induce formation of reactive oxygen species in hepatocytes (9). In addition, various studies have shown that TNF- α , a major mediator of septic shock, induces tissue injury (10). Increased level of hepatotoxic cytokines such as TNF- α are well documented in alcoholic liver disease (ALD) and nonalcoholic steatohepatitis (NASH) and have been shown to play a mechanistic role in both of these disease processes (11). Severity of liver damages has been correlated with concentration of TNF- α in alcoholic patients (12).

Activation of the TNF- α system has a pivotal role in the inflammatory process of chronic hepatitis C, and TNF- α levels correlate with the histological severity of inflammation (13). In light of role played by TNF- α in liver damage of chronic hepatitis C and insulin resistance and liver damage of HCV patients with diabetes: a novel triad of TNF- α , chronic hepatitis C and diabetes is proposed (23).

TNF- α has been shown by several studies to link obesity, a known major risk factor for type 2 DM, and insulin resistance (14-17). Expression of TNF- α mRNA was increased, and was strongly correlated to the degree of obesity and the level of insulin resistance in obese animal models and humans (14-15). Long-term exposure of animals to TNF- α induced insulin resistance, whereas neutralization of TNF- α increased insulin sensitivity (16-18),

2) Diseases associated with ROS

In osteoarthritis (OA), it has been shown in vitro and in vivo that ROS and oxidative stress contribute to cartilage degeneration (21-22). Tissue from OA patient joints stained for nitrotyrosine; a marker indicative of oxidative damage. In addition, telomere length in the patients' chondrocytes were shortened; a condition also attributed to oxidative damage. Shortening of telomeres lessens a cells' replicative capacity, in this case the cells are chondrocytes and this leads to

a senescent state. This ROS-mediated chondrocyte senescence is one path to cartilage deterioration.

Because of the role of ROS in cell signaling, an imbalance in ROS presence can influence the activity of vascular cells and play a role in pathologic conditions such as atherosclerosis and hypertension (23).

Diabetic kidney damage is closely associated with remodeling involving the extracellular matrix (ECM) and epithelial-mesenchymal transition (EMT) (19). This process is mediated by reactive oxygen species (ROS) signaling which can be induced in conditions of high glucose. Superoxide anions and hydrogen peroxide are examples of ROS that are involved in these types of destruction. Accumulation of ECM is due to an increase in synthesis coupled with a decrease in degradation leading to glomerular mesangial expansion. In conditions of EMT there is increased turnover of this accumulated ECM which leads to tubulointerstitial fibrosis and ultimately kidney damage. Signaling via ROS is key to this process and controlling ROS-mediated signaling could be a means to control and possibly inhibit the kidney remodeling. There is some evidence that oxidative stress can play a role in determining patient well-being in end stage kidney disease (20).

Referneces

1. Naguwa, S.M. 2005. Tumor necrosis factor inhibitor therapy for rheumatoid arthritis. *Ann NY Acad Sci* 1051:709-715.
2. Atzeni, F., Turiel, M., Capsoni, F., Doria, A., Meroni, P., Sarzi-Puttini, P. 2005. Autoimmunity and anti-TNF-alpha agents. *Ann NY Acad Sci* 1051:559-569.
3. Elliott, M.J., M. Feldmann, and R.N. Maini. 1995. TNF alpha blockade in rheumatoid arthritis: rationale, clinical outcomes and mechanisms of action. *Int J Immunopharmacol* 17:141-145.
4. Sands, B.E., F.H. Anderson, C.N. Bernstein, W.Y. Chey, B.G. Feagan, R.N. Fedorak, M.A. Kamm, J.R. Korzenik, B.A. Lashner, J.E. Onken, D. Rachmilewitz, P. Rutgeerts, G. Wild, D.C. Wolf, P.A. Marsters, S.B. Travers, M.A. Blank, and S.J. van Deventer. 2004. Infliximab maintenance therapy for fistulizing Crohn's disease. *N Engl J Med* 350:876-885.
5. Ferraro-Peyret, C., Coury, F., Tebib, J.G., Bienvenu, J., Fabien, N. 2004. Infliximab therapy in rheumatoid arthritis and ankylosing spondylitis-induced specific antinuclear and antiphospholipid autoantibodies without autoimmune clinical manifestations: a two-year prospective study. *Arthritis Res Ther* 6:R535-R543.
- 6.. Nash, P.T., Florin, T.H.J. 2005. Tumor necrosis factor inhibitors. *M A J* 183:205-208.
- 7.. Catrina, A.I., Lampa, J., Ernestam, S., af Klint, E., Bratt, J., Klareskog, L., Ulfgren, S.-K. 2002. Anti-tumor necrosis factor (TNF)-alpha therapy

(etanercept) down-regulates serum matrix metalloproteinase (MMP)-3 and MMP-1 in rheumatoid arthritis. *Rheumatology* 41:484-489.

- 8.. Shealy, D.J., P.H. Wooley, E. Emmell, A. Volk, A. Rosenberg, G. Treacy, C.L. Wagner, L. Mayton, D.E. Griswold, and X.Y. Song. 2002. Anti-TNF-alpha antibody allows healing of joint damage in polyarthritic transgenic mice. *Arthritis Res* 4:R7.
- 9.. Admason GM, Billings, RE, Tumor necrosis factor induced oxidative stress in isolated mouse hepatocytes, *Arch. Biochem. Biophys.* (1992), 294, 223-9..
- 10. Tracey, K.J., Cerami, A., Tumor necrosis factor: a pleiotropic cytokine and therapeutic target. *Ann. Rev. Med.* (1994), 45, 491-503.
- 11.. Song Z, Joshi-Barve S, Barve S, McClain CJ. Advances in alcoholic liver disease, *Current Gastroenterology Reports* 2004, 6:71-76.
- 12. McClain CJ, Song Z, Barve S, Hill DB, Deaciuc I. Recent Advances in Alcoholic Liver Disease IV. Dysregulated cytokine metabolism in alcoholic liver disease. *Am. J. Physio Gastrointest Liver Physiol.* 2004, 287, G497-G502.
- 13. Knobler H, Schattner A. TNF- α , chronic hepatitis C and diabetes: a novel triad. *QJM.* 2005;98:1-6.
- 14. Greenberg AS, McDaniel ML. Identifying the links between obesity, insulin resistance and b-cell function: potential role of adipocyte-derived cytokines in the pathogenesis of type 2 diabetes. *Eur J Clin Invest* 2002; 32(Suppl 3):24-34.
- 15. Ruan H, Lodish HF. Insulin resistance in adipose tissue: direct and indirect effects of tumor necrosis factor- α . *Cytokine Growth Factor Rev* 2003; 14:447-55.
- 16. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* 1993; 259:87-91.
- 17. Hotamisligil GS, Budavari A, Murray D, Spiegelman BM. Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. Central role of tumor necrosis- α . *J Clin Invest* 1994; 94:1543-9.
- 18. Lang CH, Dobrescu C, Bagby GJ. Tumor necrosis factor impairs insulin action on peripheral glucose disposal and hepatic glucose uptake. *Endocrinology* 1992; 130:43-52..
- 19. Ha, H., Lee, H.B. 2003. Reactive oxygen species and matrix remodeling in diabetic kidney. *J Am Soc Nephrol* 14:s246-s249.
- 20. Locatelli, F., Canaud, B., Eckardt, K.-U., Stenvinkel, P., Wanner, C., Zoccali, C. 2003. Oxidative stress in end-stage renal disease: an emerging threat to patient outcome. *Nephrol Dial Transplant* 18:1272-1280.
- 21. Yudoh, K., van Trieu, N., Nakamura, H., Hongo-Masuko, K., Kato, T., Nishioka, K. 2005. Potential involvement of oxidative stress in cartilage senescence and development of osteoarthritis: oxidative stress induces chondrocyte telomere instability and downregulation of chondrocyte function. *Arthritis Res Ther* 7:R380-R391.

22. Henrotin, Y.E., Bruckner, P., Pujol, J.-P.. L. 2003. The role of reactive oxygen species in homeostasis and degradation of cartilage. *OsteoArthritis and Cartilage* 11:747-755.
23. Griendling, K.K., Sorescu, D., Lassegue, B., Ushio-Fukai, M. 2000. *Arterioscler Thromb Vasc Biol* 20:2175-2183.

This Publication Is Searchable

 SEARCH

[General]

The liver is a complex organ with interdependent metabolic, excretory, and defense functions. No single or simple test assesses overall liver function; sensitivity and specificity are limited. Use of several screening tests improves the detection of hepatobiliary abnormalities, helps differentiate the basis for clinically suspected disease, and determines the severity of liver disease. Many tests are available, but relatively few improve patient care.

The Merck Manual of Diagnosis and Therapy
Section 4. Hepatic And Biliary Disorders
Chapter 37. Screening And Diagnostic Evaluation
Topics
[General]

navigation help

Laboratory Tests

Among automatic analyses, the most useful are serum bilirubin, alkaline phosphatase, and aminotransferase (transaminase). Cholesterol and LDH are less valuable. The prothrombin time indicates the severity of hepatocellular disease. Only a few biochemical and serologic tests are diagnostic by themselves (eg, hepatitis B surface antigen [HBsAg] for hepatitis B virus, serum copper and ceruloplasmin for suspected Wilson's disease, serum α_1 -antitrypsin for α_1 -antitrypsin deficiency).

Bilirubin: Hyperbilirubinemia results from increased bilirubin production, decreased liver uptake or conjugation, or decreased biliary excretion (see Jaundice in Ch. 38). Increased bilirubin production (eg, from hemolysis) or decreased liver uptake or conjugation (eg, Gilbert's disease) causes unconjugated (or free) bilirubin in serum to increase. Decreased bile formation and excretion (cholestasis) elevates conjugated bilirubin in serum, and the latter appears in urine.

The van den Bergh reaction measures serum bilirubin via fractionation. A direct reaction measures conjugated bilirubin. The addition of methanol causes a complete reaction, which measures total bilirubin (conjugated plus unconjugated); the difference measures unconjugated bilirubin (an indirect reaction).

Serum bilirubin may not be a particularly sensitive index of liver dysfunction or disease prognosis, but it is an established test. Total bilirubin is normally < 1.2 mg/dL (20 μ mol/L). The only value of fractionating bilirubin into its components is to detect unconjugated hyperbilirubinemia (present when the unconjugated fraction is > 15% of total bilirubin). Fractionation is usually required in cases of an isolated bilirubin elevation (ie, other conventional liver tests are normal) or neonatal jaundice. Sophisticated techniques to separate the various conjugates of bilirubin have no clinical value.

Urine bilirubin is normally absent. Its presence, readily detected at the bedside with a commercial urine test strip, indicates hepatobiliary disease. Unconjugated hyperbilirubin is tightly bound to albumin, not filtered by the glomerulus, and absent from urine even with raised serum levels of unconjugated bilirubin. A positive test for urine bilirubin confirms that any raised plasma levels are from conjugated hyperbilirubinemia. There is no need to fractionate the total plasma bilirubin. An early feature of hepatobiliary disease can be bilirubinuria, which develops in acute viral hepatitis even before clinical

jaundice appears. It may be absent, however, under other circumstances despite increased serum bilirubin. False-negatives occur with prolonged storage of the urine specimen, which may oxidize bilirubin, or in the presence of ascorbic acid (from vitamin C ingestion) or nitrate in the urine (from urosepsis).

Urobilinogen is normally present in trace amounts in the urine (10 mg/L [17 μ mol/L]) and can be assessed by commercial test strips. This intestinal metabolite of bilirubin becomes elevated from hemolysis (excess pigment formation) or from mildly impaired liver uptake and excretion (ie, when the enterohepatic circulation of this pigment exceeds the liver's capacity to clear and excrete it). Failure of bilirubin excretion into the small intestine reduces urobilinogen formation so that the urine may test falsely low or absent. Thus, although sensitive for mild liver disease, urobilinogen is too nonspecific and too difficult to interpret.

Alkaline phosphatases: These isoenzymes can hydrolyze organic phosphatase ester bonds in an alkaline medium, generating an organic radical and inorganic phosphate. Their biologic function is unknown.

Alkaline phosphatase in serum normally comes from the liver and bone and, during pregnancy, from the placenta. It is present in some tumors (eg, bronchogenic carcinoma). Bone growth causes an age-dependent rise in normal values, particularly in children < 2 yr and adolescents. Thereafter, alkaline phosphatase activity declines, reaching normal adult levels after a growth spurt during adolescence. It is slightly increased in older people. During pregnancy, serum levels rise two- to fourfold by the 9th mo and return to normal by 21 days' postpartum.

Alkaline phosphatase increases markedly in diseases that impair bile formation (cholestasis) and to a lesser extent in hepatocellular disease. Values in cholestasis, whether from intrahepatic causes (primary biliary cirrhosis, drug-induced liver disease, liver transplantation rejection) or graft-vs.-host disease or from extrahepatic causes (bile duct obstruction from stricture, stone, or tumor), rise similarly, up to fourfold. The elevation is not discriminatory. In hepatocellular disease (eg, various forms of hepatitis, cirrhosis, infiltrative disorders), alkaline phosphatase levels tend to be somewhat lower, although overlap exists.

Isolated elevations (ie, other liver tests are normal) occur in granulomatous hepatitis or focal liver disease (eg, abscess, neoplastic infiltration, partial bile duct obstruction). In some nonhepatic malignancies without liver metastasis, the mechanism is obscure. For example, bronchogenic carcinoma may produce its own alkaline phosphatase; hypernephroma in 15% of cases induces nonspecific hepatitis as the presumed origin of the enzyme elevation. For Hodgkin's lymphoma, the cause of the isolated alkaline phosphatase elevation is unknown. Generally, an isolated alkaline phosphatase elevation in an otherwise asymptomatic elderly adult is not worth investigating. Most cases originate from the bone (eg, in Paget's disease).

5'-Nucleotidase: Measurement of 5'-nucleotidase is simpler than available techniques that assess elevated alkaline phosphatase to distinguish bone from liver origin. 5'-Nucleotidase differs biochemically from alkaline phosphatase and is more restricted to the plasma membranes of the liver cell. Values are low in childhood, rise gradually during adolescence, and plateau after age 50 yr. 5'-Nucleotidase is normally elevated in some women during the last trimester of pregnancy. This serum enzyme increases in hepatobiliary but not in bone diseases. It is useful in assessing the anicteric patient. Because of its specificity for liver disease, 5'-nucleotidase offers some advantage over alkaline phosphatase, but neither can differentiate obstructive from hepatocellular disease. They may or may not rise and fall in parallel.

γ -Glutamyl transpeptidase (GGT): Also known as γ -glutamyltransferase, GGT (present in the liver, pancreas, and kidney) transfers the γ -glutamyl group from one peptide to another or to an L-amino acid. GGT levels are elevated in diseases of the liver, biliary tract, and pancreas when the common duct is obstructed. GGT levels parallel those of alkaline phosphatase and 5'-nucleotidase in cholestatic conditions. The extreme sensitivity of GGT (greater than that of alkaline phosphatase) limits its

usefulness, but it helps detect hepatobiliary disease as the cause of an isolated rise in alkaline phosphatase. GGT is normal in pregnancy and bone disease. Because it is not physiologically elevated in pregnancy or childhood, GGT may distinguish hepatobiliary disease in such cases. Drug use and alcohol ingestion, which induce microsomal enzymes, also elevate GGT. As a marker for alcoholic liver disease, GGT is poor when used alone but more secure when combined with transaminases.

Transaminases: Aspartate transaminase (AST) and alanine aminotransferase (ALT) are sensitive indicators of liver injury. AST is present in the heart, skeletal muscle, brain, and kidney as well as in the liver. AST levels thus rise in MI, heart failure, muscle injury, CNS disease, and other nonhepatic disorders. AST is relatively nonspecific, but high levels indicate liver cell injury. ALT is reliable for routine screening for liver disease. Values > 500 IU/L suggest acute viral or toxic hepatitis and occur with marked heart failure (ischemic hepatitis) and occasionally with common duct stones. The magnitude of the elevation has no prognostic value and does not correlate with the degree of liver damage. Serial testing provides good monitoring: A fall to normal indicates recovery unless associated with the end stages of massive hepatic necrosis.

ALT is found primarily in liver cells and thus has greater specificity for liver disease but offers little other advantage. In most liver diseases, the AST increase is less than that of ALT (AST/ALT ratio < 1), but in alcohol-related liver injury, the ratio frequently is > 2 . The basis for this is the greater need of pyridoxal 5'-phosphate (vitamin B₆) as a cofactor for ALT; this cofactor is deficient in the alcoholic, limiting the rise of ALT. Although the practicality of the ratio is limited, an AST/ALT ratio > 3 with an inordinate increase in GGT (more than twice the alkaline phosphatase) is highly suggestive of alcohol-related liver injury (eg, alcoholic hepatitis).

Lactic dehydrogenase: LDH, commonly included in routine analysis, is insensitive as an indicator of hepatocellular injury but is better as a marker for hemolysis, MI, or pulmonary embolism. LDH can be quite high with malignancies involving the liver.

Serum proteins: The liver synthesizes most serum proteins: α - and β -globulins, albumin, and clotting factors (but not γ -globulin, which is produced by B lymphocytes). Hepatocytes also make specific proteins: α_1 -antitrypsin (absent in α_1 -antitrypsin deficiency), ceruloplasmin (reduced in Wilson's disease), and transferrin and ferritin (saturated with iron and greatly increased, respectively, in hemochromatosis). These serum proteins and some others increase nonspecifically in response to tissue injury (eg, inflammation) with the release of cytokines. Such acute phase reactions may produce a spuriously normal or elevated value.

Serum albumin, the main determinant of plasma oncotic pressure, transports numerous substances (eg, unconjugated bilirubin). Its serum concentration is determined by the relative rates of its synthesis and degradation or loss, by its distribution between the intra- and extravascular beds, and by the plasma volume. In adults, the liver normally synthesizes 10 to 15 g (0.2 mmol) of albumin daily, which represents about 3% of the total body pool. Its biologic half-life is about 20 days; thus, serum levels do not reflect hepatocellular function in acute liver disease. Serum albumin (and its synthesis) is decreased in chronic liver disease (eg, cirrhosis, ascites), largely because of the increased volume of distribution. Alcoholism, chronic inflammation, and protein malnutrition depress albumin synthesis. Hypoalbuminemia can result from excess albumin loss from the kidney (nephrotic syndrome), gut (protein-losing gastroenteropathies), and skin (burns).

Serum immunoglobulins rise in most cases of chronic liver disease when the reticuloendothelial system is defective or bypassed by portal venous shunts. The inability to clear portal venous blood of transient bacteremias from normal colonic flora results in chronic antigenic stimulation of extrahepatic lymphoid tissue and hypergammaglobulinemia. Serum globulin levels rise slightly in acute hepatitis and more markedly in chronic active hepatitis, particularly of the autoimmune variety. The pattern of immunoglobulin increase adds little: IgM is quite elevated in primary biliary cirrhosis, IgA in alcoholic liver disease, and IgG in chronic active hepatitis.

Antibodies: Specific proteins may be diagnostic. Viral antigens and antibodies are associated with specific causes of hepatitis (see Acute Viral Hepatitis in Ch. 42 and Infectious Mononucleosis under Viral Infections in Ch. 265).

Antimitochondrial antibodies are directed against antigens on the inner mitochondrial membrane of several tissues. The M₂ antigen is most closely associated with primary biliary cirrhosis.

Antimitochondrial antibodies are positive, usually in high titers, in > 95% of patients with primary biliary cirrhosis. These heterogeneous antibodies are also present in 30% of cases of autoimmune chronic active hepatitis and in some cases of drug hepatitis and collagen vascular disease. They are absent in mechanical biliary obstruction and primary sclerosing cholangitis; hence, they have important diagnostic value, particularly when liver histopathology is equivocal.

Other antibodies occur in autoimmune chronic active hepatitis: Smooth muscle antibodies directed against actin are found in 70%, and antinuclear antibodies providing a homogenous (diffuse) fluorescence are positive in high titers. Some patients with chronic active hepatitis exhibit a different autoantibody, anti-liver-kidney-microsome (LKM-1) antibody. However, none of these antibodies is diagnostic by itself, and none reveals the pathogenesis of the disease process.

α-Fetoprotein (AFP): Synthesized by the fetal liver, AFP is normally elevated in the mother and newborn. By 1 yr of age, infants achieve adult values (normally < 20 ng/mL). Marked elevations develop in primary hepatocellular carcinoma; the level correlates with tumor size. AFP is a useful screening test because few other conditions (embryonic teratocarcinomas, hepatoblastomas, infrequent hepatic metastases from the GI tract, some cholangiocarcinomas) cause levels > 400 ng/mL. In fulminant hepatitis, AFP can be > 1000 ng/mL; lesser elevations (100 to 400 ng/mL) occur in acute and chronic hepatitis. These values may represent hepatic regeneration.

Prothrombin time (PT): PT involves the interactions of factors I (fibrinogen), II (prothrombin), V, VII, and X, which are synthesized by the liver (see also discussion under Hemostasis in Ch. 131). PT may be expressed in time (sec) or as a ratio of measured PT vs. control PT, termed the INR. Vitamin K is necessary for prothrombin conversion. The precursors of factors VII, IX, X, and possibly V require it for activation through a carboxylation step, which is essential for them to function as clotting factors. Vitamin K deficiencies result from inadequate intake or malabsorption. Because it is fat-soluble, vitamin K requires bile salts for intestinal absorption and would therefore be deficient in cholestasis. Malabsorption of vitamin K as a cause of a prolonged PT can be differentiated by repeating the PT 24 to 48 h after administration of vitamin K 10 mg sc. Little or no improvement occurs with parenchymal liver disease.

PT is relatively insensitive for detecting mild hepatocellular dysfunction. Because the biologic half-lives of the involved clotting factors are short (hours to a few days), the PT has a high prognostic value in acute liver injury. In acute viral or toxic hepatitis, PT > 5 sec above control is an early indicator of fulminant hepatic failure.

Tests for hepatic transport and metabolism: Several tests can determine the ability of the liver to transport organic material and metabolize drugs. Bilirubin measurements are common; other tests, although often very sensitive, are complex, costly, and nonspecific.

Bile acids are specific to the liver, being synthesized only in the liver, constituting the driving force for bile formation and exhibiting a 70 to 90% first-pass hepatic extraction. Serum bile acid concentrations normally are extremely low (about 5 μmol/L). Elevations are specific and very sensitive for hepatobiliary disease, but they do not assist in differential diagnosis nor indicate prognosis. Values are normal in isolated hyperbilirubinemia (eg, Gilbert's syndrome). Sophisticated analysis of individual serum bile acids may aid clinical research of bile acid therapy for gallstone disease and primary biliary cirrhosis.

Imaging Studies

Radionuclide scanning, ultrasound (US), CT, and MRI have replaced traditional imaging techniques (eg, oral cholecystogram, IV cholangiogram). Invasive radiography (eg, ERCP) allows for sophisticated instrumentation and treatment procedures.

Plain x-ray of the abdomen: The usefulness of x-rays is limited to identifying calcifications in the liver or gallbladder, opaque gallstones, and air in the biliary tract. Hepatic or splenic enlargement and ascites may be detected.

Oral cholecystogram: This procedure is simple, reliable, and relatively safe for visualizing the gallbladder; 25% of patients experience diarrhea. Rarely, a patient has a hypersensitivity reaction to the iodine in the contrast agent. An abnormal study includes failure to visualize the gallbladder after a second dose of contrast agent, provided the obvious has been excluded: vomiting, gastric outlet obstruction, malabsorption, Dubin-Johnson syndrome, and significant hepatocellular disease. Sensitivity for diagnosing gallbladder disease (eg, cholelithiasis) is about 95%, but specificity is much lower. Conversely, gallstones and tumors are readily identified and differentiated. Besides defining gallbladder anatomy, oral cholecystography also assesses the patency of the cystic duct and, to a lesser extent, the concentrating function of the gallbladder. Radiologic gallbladder filling is an important criterion when assessing patients for gallstone dissolution therapy with bile salts and for biliary lithotripsy. This technique is also more useful than US for determining stone number and type (lucency implies that the stones are composed of cholesterol). However, US and biliary cholescintigraphy have largely replaced this former gold standard because of their greater ease of use and lower false-negative rates. Cholescintigraphy is also better at assessing gallbladder filling and emptying.

Ultrasound: Findings obtained by US are morphologic and independent of function. US is the most important investigative tool in screening for biliary tract abnormalities and mass lesions in the liver. US is better at detecting focal lesions (> 1 cm in diameter) than diffuse disease (eg, fatty liver, cirrhosis). In general, cysts are echo-free; solid lesions (eg, tumors, abscesses) tend to be echogenic. The ability to localize focal lesions permits US-guided aspiration and biopsy.

US is the least expensive, safest, and most sensitive technique for visualizing the biliary system, especially the gallbladder. Accuracy in detecting gallbladder or gallstone disease is close to 100%, although an element of operator skill is needed. Gallstones cast intense echoes with distal shadowing and may move with gravity. Size can be accurately defined, but the number of stones may be difficult to determine because of overlap when many are present. Criteria for acute cholecystitis include a thickened gallbladder wall, pericholecystic fluid, an impacted stone in the gallbladder neck, and gallbladder tenderness on palpation (Murphy's sign). Polyps of the gallbladder are a frequent incidental finding. Carcinoma presents as a nonspecific solid mass.

US is the procedure of choice for evaluating cholestasis and differentiating extrahepatic from intrahepatic causes of jaundice. Bile ducts stand out as echo-free tubular structures. The diameter of the common duct is normally < 6 mm, increases slightly with age, and can reach 10 mm after cholecystectomy. Dilated ducts are virtually pathognomonic for extrahepatic obstruction, but normal bile ducts do not exclude obstruction because it may be recent or intermittent. US does not readily detect common duct stones, but they may be inferred if the common duct is dilated and stones are identified in the gallbladder. Visualization of the pancreas, kidney, and blood vessels is an added bonus. Finding enlargement or a mass in the head of the pancreas may reveal the cause of cholestasis or upper abdominal pain.

Doppler US measures the frequency change of a backscattered US wave reflected from moving RBCs. This can show the patency of hepatic vessels, particularly the portal vein, and the direction of blood flow. Doppler US can reveal hepatic artery thrombosis after liver transplantation. It also can detect unusual vascular structures (eg, cavernous transformation of the portal vein).

Radionuclide scanning: This procedure involves hepatic extraction of an injected

- radiopharmaceutical from the blood, most commonly technetium 99m (^{99m}Tc).
- Liver-spleen scanning uses ^{99m}Tc -sulfur colloid, which is rapidly extracted from the blood by reticuloendothelial cells. Normally, radioactivity is uniformly distributed. In a space-occupying lesion > 4 cm (eg, cyst, abscess, metastasis, hepatic tumor), the replaced liver cells produce a cold spot. Generalized liver disease (eg, cirrhosis, hepatitis) causes a heterogeneous decrease in liver uptake and increased uptake by the spleen and bone marrow. In hepatic vein obstruction, there is decreased visualization of the liver except for the caudate lobe because of its special drainage into the inferior vena cava. US or CT has largely supplanted radionuclide scanning for space-occupying lesions and diffuse parenchymal disease.

Cholescintigraphy: For scanning the hepatobiliary excretory system, cholescintigraphy uses ^{99m}Tc -imino diacetic acid derivatives. These radiopharmaceuticals are organic anions, which the liver avidly clears from plasma into bile much like bilirubin. A minimum 2-h fast is necessary. A normal scan shows rapid, uniform liver uptake; prompt excretion into the bile ducts; and a visible gallbladder and duodenum by 1 h. In acute cholecystitis (with cystic duct obstruction), the gallbladder is not visible by 1 h. Acute acalculous cholecystitis can similarly be detected. Chronic cholecystitis is more problematic: It can be reasonably diagnosed if gallbladder visualization is delayed beyond 1 h, sometimes until 24 h, or if the gallbladder is never visualized, but confounded by false-negatives and false-positives. Several factors may contribute to nonvisualization of the gallbladder (eg, significant cholestasis with markedly elevated bilirubin, a nonfasting state, fasting > 24 h, certain drugs).

Cholescintigraphy also assesses hepatobiliary integrity (bile leaks may be especially important after surgery or trauma) and anatomy (from congenital choledochal cysts to choledochoenteric anastomoses). After cholecystectomy, this biliary scan can quantitate biliary drainage and assist in defining sphincter of Oddi dysfunction. In neonatal jaundice, hepatobiliary imaging helps distinguish hepatitis from biliary atresia.

Computed tomography: CT is sensitive to variations in density of differing hepatic lesions. The addition of an IV contrast agent helps differentiate more subtle differences between soft tissues and define the vascular system and the biliary tract. CT shows liver structures more consistently than US; neither obesity nor intestinal gas obscures them. CT is especially useful for visualizing space-occupying lesions (eg, metastases) in the liver and masses in the pancreas. CT can detect fatty liver and the increased hepatic density associated with iron overload. CT is expensive and necessitates radiation exposure; both factors lessen its routine use compared with US.

Magnetic resonance imaging: MRI is an exciting, although expensive, technology that may prove advantageous for identification of tumors and hepatic blood flow. Blood vessels are easily identified without contrast agents. Although still evolving, MRI is comparable to CT for detecting mass lesions and can visualize perihepatic vessels and the biliary system. Magnetic resonance cholangiography is becoming an increasingly useful screening tool before proceeding to more invasive techniques.

Operative cholangiography: This procedure entails direct injection of a contrast agent into the cystic duct or common bile duct at laparotomy. Excellent visualization results. This diagnostic approach is indicated for biliary stones when jaundice occurs or when a common duct stone is suspected. Technical difficulties have limited its use at laparoscopic cholecystectomy. Direct visualization of the common duct can also be obtained by choledochoscopy. **IV cholangiography** for identifying the common duct has been virtually abandoned because of poor diagnostic yield, the risk of a hypersensitivity reaction, and the advent of ERCP.

Endoscopic retrograde cholangiopancreatography: ERCP combines (1) endoscopy (for upper GI endoscopy, see Ch. 19) for identifying and cannulating the ampulla of Vater in the second portion of the duodenum and (2) radiology after injection of a contrast agent into the biliary and pancreatic ducts. This technique places a side-viewing endoscope in the descending duodenum, identifies and cannulates the papilla of Vater, and then injects a contrast agent to visualize the pancreatic duct and the biliary duct

systems. Besides obtaining excellent images of the biliary tract and pancreas, ERCP allows some visualization of the upper GI tract and the periampullary area. Biopsies and interventional procedures may be performed (eg, sphincterotomy, biliary stone extraction, placement of a biliary stent in a stricture). ERCP is an outpatient procedure that, in experienced hands, has relatively low risk (mainly pancreatitis in 3% after sphincterotomy). It has revolutionized the diagnosis and management of pancreaticobiliary disease. ERCP is especially valuable in assessing the biliary tract in cases of persistent jaundice and in seeking a lesion amenable to intervention (eg, stone, stricture, sphincter of Oddi dysfunction). In jaundice and cholestasis, US to assess duct size should precede ERCP.

Percutaneous transhepatic cholangiography (PTC): This procedure involves puncture of the liver with a 22-gauge needle under fluoroscopic or US control to enter the peripheral intrahepatic bile duct system above the common hepatic duct. PTC has a high diagnostic yield but only for the biliary system. Some therapeutic techniques (eg, decompression of the biliary system, insertion of an endoprosthesis) are possible. ERCP generally is preferred, particularly if ducts are not dilated (eg, sclerosing cholangitis). PTC is used after failed ERCP or when altered anatomy (gastroenterostomy) precludes accessing the ampulla. It may complement ERCP in hilar lesions at the porta hepatis. PTC is generally safe but has a higher complication rate (eg, from sepsis, bleeding, bile leaks) than ERCP. Local expertise often dictates the choice between PTC and ERCP.

Liver Biopsy

Percutaneous liver biopsy provides valuable diagnostic information with relatively small risk and little patient discomfort. Performed with the patient under local anesthesia, this bedside procedure entails aspiration (using the Menghini needle or the disposable and therefore always sharp Jamshidi needle) or cutting (using the disposable Trucut--a variation of the Vim-Silverman needle). The needle is inserted through an anesthetized intercostal space anterior to the midaxillary line, just below the point of maximal dullness on expiration. The patient lies still and maintains expiration. The liver is rapidly entered with either suction applied (Jamshidi) or a cutting sheath advanced (Trucut). The result is a procedure that takes 1 to 2 sec and yields a liver specimen 1 mm in diameter and 2 cm long. Occasionally, a second pass is necessary; if a second or third attempt is unsuccessful, then needle biopsy should be guided by ultrasound (US) or CT. US-guided biopsies using a biopsy gun, whose spring mechanism fires a modified Trucut needle, are less painful and provide a high yield. US guidance is particularly useful for sampling focal lesions or avoiding vascular lesions (eg, hemangiomas).

At biopsy, the liver's texture can be ascertained on needle insertion: a hard, gritty feel suggests cirrhosis. The biopsy is examined routinely for histopathology. Cytology, frozen section, and culture may be useful in selected cases. In suspected Wilson's disease, copper content should be measured. Gross inspection provides information: fragmentation suggests cirrhosis; a fatty liver is pale yellow and floats in formaldehyde; carcinoma is whitish.

Liver biopsy is sufficiently safe to perform as an outpatient procedure. After biopsy, the patient is monitored for 3 to 4 h, during which complications (eg, intra-abdominal hemorrhage, bile peritonitis, lacerated liver) are most likely. Because delayed bleeding can occur as long as 15 days later, discharged patients should remain within 1 h of the hospital. Mild right upper quadrant discomfort, sometimes radiating from the diaphragm to the shoulder tip, is common and responds to mild analgesics. Mortality is low at 0.01%; major complications are reportedly about 2%.

Indications for percutaneous liver biopsy are listed in [Table 37-1](#). Fine-needle biopsy under US guidance detects metastatic carcinoma in at least 66% of cases and may establish the diagnosis despite negative scanning techniques; cytologic examination of the biopsy fluid yields positive findings in an additional 10% of cases. Results are less valuable in lymphoma and correlate poorly with the clinical impression of hepatic involvement. Biopsy is especially valuable in detecting TB or other granulomatous infiltrations and can clarify graft problems (ischemic injury, rejection, biliary tract disease, viral hepatitis) after liver transplantation.

Limitations of the procedure include (1) the need for a skilled histopathologist (many pathologists have little experience with needle specimens); (2) sampling error (nonrepresentative tissue seldom occurs in hepatitis and other diffuse conditions but can be a problem in cirrhosis and space-occupying lesions); (3) inability to differentiate hepatitis etiologically (eg, viral vs. drug-induced); and (4) occasional errors or uncertainty in cases of cholestasis.

Relative contraindications include a clinical bleeding tendency or a coagulation disorder (prothrombin time > 3 sec over control values [INR > 1.2] despite giving vitamin K, bleeding time > 10 min), severe thrombocytopenia (50,000/ μ L), severe anemia, peritonitis, marked ascites, high-grade biliary obstruction, and subphrenic or right pleural infection or effusion.

Transvenous liver biopsy is performed by threading a modified Trucut needle through a catheter inserted into the right internal jugular vein and through the right atrium into the inferior vena cava and hepatic vein. The needle is advanced through the hepatic vein into the liver. Hepatic vein and wedge pressures can also be obtained. Although the specimen obtained is relatively small and the operator must be skilled in angiography, this technique can be used even when the patient has a significant coagulation disorder. It is surprisingly well tolerated and requires minimal sedation, if any, except in the case of an uncooperative patient. The yield for liver tissue is > 95% in experienced hands. The complication rate is very low: 0.2% bleed from puncture of the liver capsule. One center reported no mortality in > 1000 transvenous biopsies.